Two atypical forms of HbH disease in Sardinia

by Maria Elisabetta Paglietti, Maria Carla Sollaino, Daniela Loi, Francesca Sarra, Eleonora Zaccheddu, and Renzo Galanello

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Hemoglobin H disease is usually caused by deletion or inactivation of three α globin genes, leaving intact and active only one α-globin gene (1). The most frequent defects responsible for HbH disease in Sardinia are the coinheritance of the -α3.7 Kb deletion or, less frequently, the α2 initiation codon mutation ATG>ACG (α2Ncol) in the other chromosome (2,3). HbH disease due to deletions including the major upstream regulatory element (MCS-R2) and leaving intact both α-globin genes, have also been described (4,5). We report here two new α0 deletions, both located on the short arm of chromosome 16, responsible for HbH disease in two different Sardinian families. These unusual deletions were respectively associated with the common α2Ncol mutation and -α3.7 deletion in trans.

Table 1 shows the hematological and molecular data of the probands and their family members. All patients had severe microcytic anemia (Hb 2.6-9.5 g/dl, MCV 52.0-75.7 fl). Jaundice, spleen enlargement, sporadic haemolytic and aplastic crisis, due to B19 parvovirus infection, requiring red blood cell transfusions, were detected in patients II-1 and II-2 of family A. A mild thalassemia-like facies was present only in II-1 of the same family. Molecular screening for the most common α-globin gene deletion and non-deletion defects, revealed the apparent homozygosity for the α2Ncol mutation in the proband of family A and in her sister and the apparent homozygosity for the -α3.7 deletion in the proband of family B. In spite of that, the α2Ncol mutation was present only in the father of family A proband and the -α3.7 deletion was present only in the mother of family B proband.

In family A MLPA analysis, carried out using MLPA kit (HBA140-B3 MCR-Holland), revealed a deletion at least of 7535 bp beginning in the region between α1-pseudo-globin gene and α2-globin gene and extending to 2.4 kb downstream α1-globin gene in the proband, in her sister and in their mother. Sequencing analysis of a ~500 bp breakpoint fragment, obtained using specific primers around MLPA deleted probes, allowed us to define the exact deletion breakpoint at position 161276/7 (5’) and 170485/6 (3’). This deletion removed a region of 9209 nt involving both α-globin genes and part of the first exon of θ gene. In addition an insertion of six nucleotides (ATTAGT) at position 161216 before the 5’ breakpoint was detected. No orphan sequence was found. The 5’ breakpoint is shifted 2 nt up and the 3’ breakpoint is shifted.
1032 nt down, as compared to the breakpoints of the α₀ thalassemia deletion found in a Dutch family recently reported (6).

In family B MLPA analysis revealed a larger deletion which removes all the MLPA probes specific for the subtelomeric region, including α-globin gene cluster with all regulatory elements, in the proband and in her father. CGH-array analysis with oligonucleotides (8x60K Agilent Technologies) and SNP genotyping allowed us to define the 3’ breakpoint between the 4th exon of NME4 gene and the IVSII of DECR2 gene (389660 and 395647 coordinates) (Figure 1).

The greater severity of the α²NcoI non deletion defect as compared to the -α³.⁷ deletion in trans to α₀ deletions, can be the reason for the different phenotypes in HbH patients of the two families (2). The different size of α₀ deletions and the loss of genes located in the deleted region in family B do not seem to interfere in the determination of specific phenotype.

Several large deletions involving α-globin gene cluster have been recently described (7-10). Although these deletions remove also other genes, affected heterozygotes appear phenotypically normal, apart from α-thalassemia carrier phenotype; however a HbH patient with a telomeric deletion of ~285 kb associated with the common -α³.⁷ deletion in trans, presented scoliosis, whose severity remains unexplained (8). A region on chromosome 16p for which haploinsufficiency leads to mental retardation typical of ATR16 has been narrowed down to a region ~0.9 and 1.5-1.7 Mb from telomere. Alu-family repeats, frequent in the genome and particularly common in and around the α-globin gene cluster, facilitate DNA strand exchanges during replication and non-homologous recombinations which are a frequently cause of α₀ deletions (9 -11). In addition to the common conventional molecular techniques, the recent alternative methods, such as MLPA and CGH, become essential for a correct α-globin genotype definition. The exact identification of uncommon and unknown alpha deletion defects, although rare, allows to offer the appropriate genetic counselling to couples at risk for HbH disease or haemoglobin Bart’s hydrops foetalis syndrome, especially in Sardinia were small isolated communities are still present.
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Authorship and disclosures
MCS and MEP wrote the manuscript. MCS, MEP and DL performed the MLPA and PCR assay. FS performed the CGH Array Analysis. EZ collected the clinical data. RG coordinates the study and approved the final manuscript. The authors reported no potential conflicts of interest.
References


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**Figure 1.** Schematic representation of the short arm of chromosome 16 (16p13.3) and of the $\alpha^0$ deletions in the two families. The $\alpha$-globin regulatory region (MCS-R 1 to 4) is indicated as black dots. Black bars represent deleted DNA regions. White bar represents the region of uncertainty for deletion breakpoint. In family-B the different methods used to detect the deletion are indicated into the boxes. SNP genotyping: loss of heterozygosity at 389660 chromosome position (rs 14293) and presence of heterozigosity at 395647 chromosome position (rs 873477) were detected. The chromosome positions of SNPs are according to GeneBank NT_010393.16.
FIGURE 1